

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

85. (Amended) A plant or plant part, which plant or plant part comprises a plant cell containing the polynucleotide according to claim 74, wherein said polynucleotide is heterologous.

*JM
Cont'd*

IN THE ABSTRACT:

After page 120, insert the Abstract of the Disclosure submitted herewith on a separate sheet.

REMARKS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

The specification has been amended to include the heading "Brief Description of the Drawings", to include sequence identifiers and to remove the hyperlink at page 85. The Examiner appears to contend that various of the sequences recited in the application do not appear in the Sequence Listing. It is believed that with the insertion of the sequence identifiers it will be clear that such is not the case.

The claims have been revised to define the invention with additional clarity. The revisions are believed to moot the rejection under 35 USC 112, second paragraph, and

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

reconsideration is requested. Claims directed at non-elected subject-matter have been cancelled.

An Abstract of the Disclosure has been provided.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "Version With Markings To Show Changes Made."

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,
NIXON & VANDERHYE, P.C.

By Mary J. Wilson
Mary J. Wilson
Reg. No. 32,955

MJW:tat

1100 North Glebe Road
8th Floor
Arlington, Virginia 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph beginning at page 19, line 1:

25L 5'-GTG CAT CTG CGT GTG CGT A-3' (SEQ ID NO:57)
25LN 5'-GTG TGC GTA CCT GGT AGA G-3' (SEQ ID NO:58)
25R 5'-AAC GAC GTC TGG TGC GTG-3' (SEQ ID NO:59)
33 5'-TGC AGC TAT ATG ACC TTC CCC CTC-3' (SEQ ID
NO:60)
37 5'-GGA CAT GCT GAT GGC TCA GA-3' (SEQ ID NO:61)
38 5'-CAG AAC TTG TCT CAT CCC TG-3' (SEQ ID NO:62)
38A 5'-GGC TAT ACA TTG GGA CTA ACA-3' (SEQ ID NO:63)
38B 5'-CGA ATC ATC ACA TCC TAT GTT-3' (SEQ ID NO:64)
39 5'-GCA AGT TCG ACT TCC AC-3' (SEQ ID NO:65)
39A 5'-TCG ACT TCC ACA AGT ACA TCA-3' (SEQ ID NO:66)
53 5'-AGC GTA CCT GCG TAC GTA G-3' (SEQ ID NO:67)

The paragraph beginning at page 30, line 21:

By way of example for nucleic acid testing, the barley *mlo-5* resistance allele is characterized by a G- to A-nucleotide substitution in the predicted start codon of the *Mlo* gene (Table 1). The mutation may easily be detected by standard PCR amplification of a *Mlo* gene segment from genomic template DNA with the primers:

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

forward primer: 5'-GTTGCCACACTTGCCACCG-3' (SEQ ID NO:68)
reverse primer: 5'-AAGCCAAGACGACAATCAGA-3' (SEQ ID NO:69)
(for example), followed by digestion [witht he] with the
restriction enzyme *Psh*A1. This generates a cleaved
amplified polymorphic sequences (CAPS) marker which may be
displayed using conventional agarose gel electrophoresis.
Presence of a 769 bp fragment is indicative of the presence
of the *mlo-5* allele.

The paragraph beginning at page 31, line 7:

The *mlo-9* resistance allele is characterized by a C-
to T- nucleotide substitution (Table 1). This allele is of
particular relevance since it is used frequently in
breeding material. The mutational event may be easily
detected using the primers:

forward primer 5'-GRRGCCACACTTGCCACG-3' (SEQ ID
NO:70)

reverse primer 5'-AAGCCAAGACGACAATCAGA-3' (SEQ ID
NO:71)

(for example) and subsequent digestion of genomic
amplification products with the restriction enzyme *Hha*I.
This generates a CAPS marker which may be displayed by
conventional agarose gel electrophoresis. The presence of
a 374 bp fragment is indicative of the presence of *mlo-9*.

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

The paragraph beginning at page 62, line 21:

Figure 2 shows an *Mlo* coding sequence and encoded amino acid sequence according to the present invention (SEQ ID NOS:1 and 2). The amino acid sequence predicted from DNA sequences of RT-PCR products from Ingrid *Mlo* are shown. Nucleotide numbers are given according to translational start site.

The paragraphs beginning at page 64, line 14:

Figure 5 shows an alignment of genomic sequences covering the barley *Mlo* gene and a rice homologue isolated via crosshybridization with a barley gene specific probe (SEQ ID NOS:3 and 4). The top line shows the barley *Mlo* genomic DNA sequence (exon sequences underlined). The bottom line shows the rice genomic sequence containing the rice *Mlo* homologue.

Figure 6 shows an alignment of genomic sequences carrying the barley *Mlo* gene and a barley homologue isolated via crosshybridization with a barley gene specific probe (SEQ ID NOS:5 and 6). The top line shows the barley *Mlo* genomic DNA sequence (exon sequences underlined). The bottom line shows the genomic sequence containing the barley *Mlo* homologue.

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

Figure 7 Nucleotide and Deduced Amino Acid Sequence of the Barley *Mlo* cDNA (SEQ ID NOs:7 and 8). The nucleotide and the deduced amino acid sequence are based on the combined data of RT-PCR and RACE obtained from experiments using RNA of cultivar Ingrid *Mlo*. The stop codon is marked by an asterisk, the putative polyadenylation signal is underlined and the detected termini of RACE products are indicated by arrows above the sequence. Positions of introns as identified by comparison with corresponding genomic clones are labelled by triangles below the nucleic acid sequence. Six predicted transmembrane spanning helices according to the MEMSAT algorithm (Jones et al., 1994) are boxed in grey colour. A putative nuclear localization signal (K-K-K-V-R) and casein kinase II site (S-I-F-D) in the carboxy-terminal half of the protein are shown in bold type.

The paragraphs beginning at page 65, line 14:

Figure 8 shows genomic sequence of rice (*Oryza sativa*) homologue including coding and flanking sequences (SEQ ID NO:9).

Figure 9 shows genomic sequence of barley (*Hordeum vulgare*) homologue including coding and flanking sequences (SEQ ID NO:10).

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

Figure 10 shows cDNA sequence of rice homologue (SEQ ID NO:11).

Figure 11 shows cDNA sequence of barley homologue (SEQ ID NO:12).

Figure 12 shows cDNA sequence of Arabidopsis thaliana homologue (SEQ ID NO:13).

Figure 13 shows amino acid sequence of rice homologue (SEQ ID NO:14).

Figure 14 shows amino acid sequence of barley homologue (SEQ ID NO:15).

Figure 15 shows amino acid sequence of Arabidopsis homologue (SEQ ID NO:16).

Figure 16 shows a pretty box of amino acid sequences of Mlo, barley, rice and Arabidopsis homologues (SEQ ID NOs:17-19).

The paragraph beginning at page 85, line 8:

A compilation of the mlo mutants and their mother varieties analyzed in this study has been described by Jørgensen (1992) [mlo-1, mlo-3, mlo-4, mlo-5, mlo-7, mlo-8, mlo-9, mlo-10, mlo-11] and by Habekuss and Henrich (1988) [mutants in cultivar Plena 2018 (mlo-13), 2034 (mlo-17), 2118]. Since mutant 2118 has not been assigned to an allele number so far, we designate the allele here as mlo-

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

26, according to current numbering in the GrainGene database (gopher colon forward slash forward slash greengenes dot cit dot cornell dot edu colon 70 forward slash 77 forward slash dot graingenes dot ndx forward slash index question mark mlo) [(gopher://greengenes.cit.cornell.edu:70/77/.graingenes.ndx/index?mlo)] .

The paragraph beginning at page 89, line 24:

RT-PCR was performed using the SUPERSCRIPT preamplification system for first strand cDNA synthesis (Gibco BRL). Total RNA (1 µg) of seven-day-old primary barley leaves (cultivar Ingrid) served as template. First strand cDNA synthesis was primed by an oligo(dT) primer. The putative coding region of the *Mlo* gene was subsequently amplified using oligonucleotides 25L (GTGCATCTGCGTGTGCGTA) (SEQ ID NO:72) and 38 (CAGAAACTTGTCTCATCCCTG) (SEQ ID NO:73) in a single amplification step (35 cycles, 60°C annealing temperature). The resulting product was analyzed by direct sequencing. 5'- and 3'-ends of the *Mlo* cDNA were determined by RACE (Frohman et al., 1988) using the MARATHON cDNA amplification kit (Clontech). Corresponding experimental procedures were mainly carried out according to the instructions of the manufacturer. To obtain specific RACE products, two consecutive rounds of

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

78. (Amended) [A] The isolated polynucleotide according to claim 74 operably linked to a regulatory sequence for expression.

79. (Amended) An isolated polynucleotide which has at least about 600 contiguous nucleotides of [the] a nucleotide sequence selected from the group consisting of the nucleotide sequence of claim 74 [or the complement thereof] and the complement of the nucleotide sequence of claim 74.

80. (Amended) [A] The isolated polynucleotide according to claim 79 operably linked to a regulatory sequence for transcription.

81. (Amended) An isolated polynucleotide which has at least about 300 contiguous nucleotides of [the] a nucleotide sequence selected from the group consisting of the nucleotide sequence of claim 74 [or the complement thereof] and the complement of the nucleotide sequence of claim 74, wherein said polynucleotide is operably linked to a regulatory sequence for transcription.

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

82. (Amended) [A] The isolated polynucleotide according to claim 74 wherein the regulatory sequence comprises an inducible promoter.

83. (Amended) A [nucleic acid] plant expressible vector [suitable for transformation of a plant cell and] comprising a polynucleotide according to claim 74.

84. (Amended) A plant cell containing [a heterologous] the polynucleotide according to claim 74,
wherein said polynucleotide is heterologous.

85. (Amended) A plant or plant part, which plant or plant part comprises a plant cell containing [a heterologous] the polynucleotide according to claim 74,
wherein said polynucleotide is heterologous.

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

932185), number T22146 (definition 4153 *Arabidopsis thaliana* cDNA clone 97N9T7, NCBI Seq ID 932186), number N37544 (definition 18771 *Arabidopsis thaliana* cDNA clone 205N12T7, NCBI Seq ID 1158686), number T88073 (definition 11769 *Arabidopsis thaliana* cDNA clone 155I23T7, NCBI Seq ID 935932) number H76041 (definition 17746 *Arabidopsis thaliana* cDNA clone 193P6T7, NCBI seq ID 1053292), number D24287 (rice cDNA partial sequence R1638_1A, nID g428139) and D24131 (rice cDNA partial sequence R1408_1A, nID g427985) are shown. The *Arabidopsis* sequences are from Newman et al. (1994) *Plant Physiol.* 106 1241-55. The rice sequences are from Minobe, Y. and Sasaki, T. submitted 2 Nov 1993 to DDBJ.

IN THE CLAIMS:

74. (Amended) An isolated polynucleotide encoding a polypeptide which comprises the amino acid sequence [shown in] of [SEQ ID NO: 2] SEQ ID NO:1.

75. (Amended) [A] The isolated polynucleotide according to claim 74 wherein the coding sequence comprises [is] the coding sequence [shown in] of [SEQ ID NO 1] SEQ ID NO:2.

SCHULZE-LEFERT et al -- Serial No.: 09/722,377

amplification (35 cycles, 55°C annealing temperature) were necessary. For this purpose, two sets of nested primers were used in combination with the adapter primers of the kit: oligonucleotides 46 (AGGGTCAGGATGCCAC) (SEQ ID NO:74) and 55 (TTGTGGAGGCCGTGTTCC) (SEQ ID NO:75) for the 5'-end and primers 33 (TGCAGCTATATGACCTCCCCCTC) (SEQ ID NO:76) and 37 (GGACATGCTGATGGCTCAGA) (SEQ ID NO:77) for the 3'-end. RACE products were subcloned into pBluescript SK (Stratagene). Ten 5'-end and eight 3' end clones were chosen for DNA sequence analysis.

The paragraphs beginning at page 91, line 24:

Table 5A show amino acid sequences, with "query" indicating part of the Mlo protein sequence to which homology has been found, with the predicted amino acid sequence of each identified EST marked with "subject" (SEQ ID NOS:20-49).

The paragraph beginning at page 92, line 1:

Table 5B shows EST nucleotide sequences encoding the amino acid sequences shown in Table 5A (SEQ ID NOS:50-56). GenBank Accession number T22145 (definition 4153 Arabidopsis thaliana cDNA clone 97N8T7, NCBI Seq ID